THE IMPLICATIONS OF *Alexandrium tamarense* RESTING CYSTS IN AN AREA OF SHELLFISH AQUACULTURE IN IRELAND.

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Abstract:
The Irish Marine Institute's Fisheries Research Centre carry out a monitoring programme for the detection of algal toxins in shellfish. This programme is carried out under EU Directive 91/492. During the course of this programme the North Channel area of Cork Harbour has been the only location in Ireland where toxins causing Paralytic Shellfish Poisoning (PSP) have been detected in shellfish above the regulatory limit.

For short periods during each of the summers of 1996, 1997 and 1998, PSP toxins were found in mussels (*Mytilus edulis*) from this area above the regulatory limit period necessitating a ban on harvesting. Oysters (*Crassostrea gigas*) from the same area remained below the regulatory threshold. The dinoflagellate *Alexandrium tamarense*, a known vector of PSP toxins, was observed in the area during each of the toxic events. The exact origin of the populations of *A. tamarense* was unknown.

A. tamarense is known to produce a cyst stage as part of its life cycle. These cysts can remain viable in the sediments for several years. A survey of the distribution of cysts of *A. tamarense* in the surface sediments in Cork Harbour was carried out in order to determine if they were potentially seeding the area. They were detected in 6 sites, and successfully germinated to yield vegetative cells. The results of the survey are presented and discussed.

Introduction:
Ireland has a recently established mariculture industry that can for the most part be attributed to the abundance of unpolluted and sheltered sites (fig 1). In light of the suitability and presence of these sites, the increase in mariculture has been dramatic in the past two decades. Unfortunately occasional losses of farmed salmon and sea trout, and an almost annual closure of shellfish production due to shellfish toxicity have marred the success of this new industry. These problems have been attributed to the presence, and in some cases blooms, of harmful dinoflagellate species.

Blooms of dinoflagellates are a frequent phenomenon in the coastal waters of Ireland (Parker *et al.* 1982, Raine *et al.* 1990). It is likely that cysts have an important role in the establishment of blooms of some species. During a two year period (1995 - 1997) a scoping study of the resting stages of dinoflagellates in Irish coastal waters was undertaken (Silke 1998). The aims of this study were to look at the distribution of these cyst stages, to see if there were any potentially toxic cyst populations in areas of mariculture and to observe the interactions between the motile and resting stages of dinoflagellates.

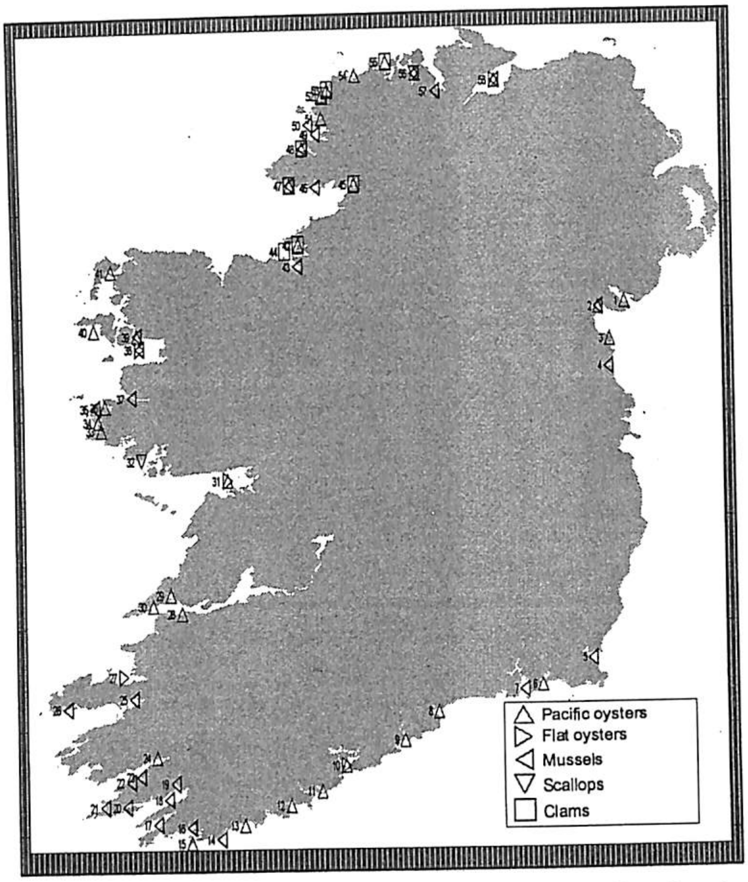


Fig 1: Major areas of shellfish production in Ireland (After Stapleton 1996)

While palynological studies by Reid (1972) had included coastal Irish sites, this present work was the first study to look at species of dinoflagellate cysts using biological methods. Of the approximate 90 known species from 29 genera of marine dinoflagellates that produce resting cysts world-wide (Nehring 1994a), 27 species of cysts from 8 named genera were recorded. The number of species found per sample ranged from 2 to 10. This compares well with the results of other biological dinoflagellate cyst studies in European waters; Lewis (1985), Ellegaard *et al.* (1994), Nehring (1994b).

This study determined that Cork Harbour in the South of the country, was the only site that had significant concentrations of the PSP causative organism *Alexandrium tamarense* both in the vegetative and cyst stages. Mussels (*Mytilus edulis*) from this area, which are routinely tested, were positive for PSP during the summers of 1996, 1997 and 1998, and this resulted in short closures of the area. No other species of shellfish tested were above 80µg/100g.

As this is an important aquaculture area for oysters of both the *Crassostrea gigas* and *Ostrea edulis* species a further survey of the area was conducted in 1999 in an attempt to determine the extent of this cyst bed.

Methods:
During the 1995 –1997 scoping study sediment samples were taken at offshore sites in the Irish Sea, Celtic Sea and the Western Irish Shelf. Samples were also taken at near-shore sites in Cork Harbour, Roaringwater Bay, Bantry Bay and Killary Harbour, which are nationally important shellfish production areas. Further near-shore samples were taken from Dingle Bay, Lough Hyne, and Belfast Lough.

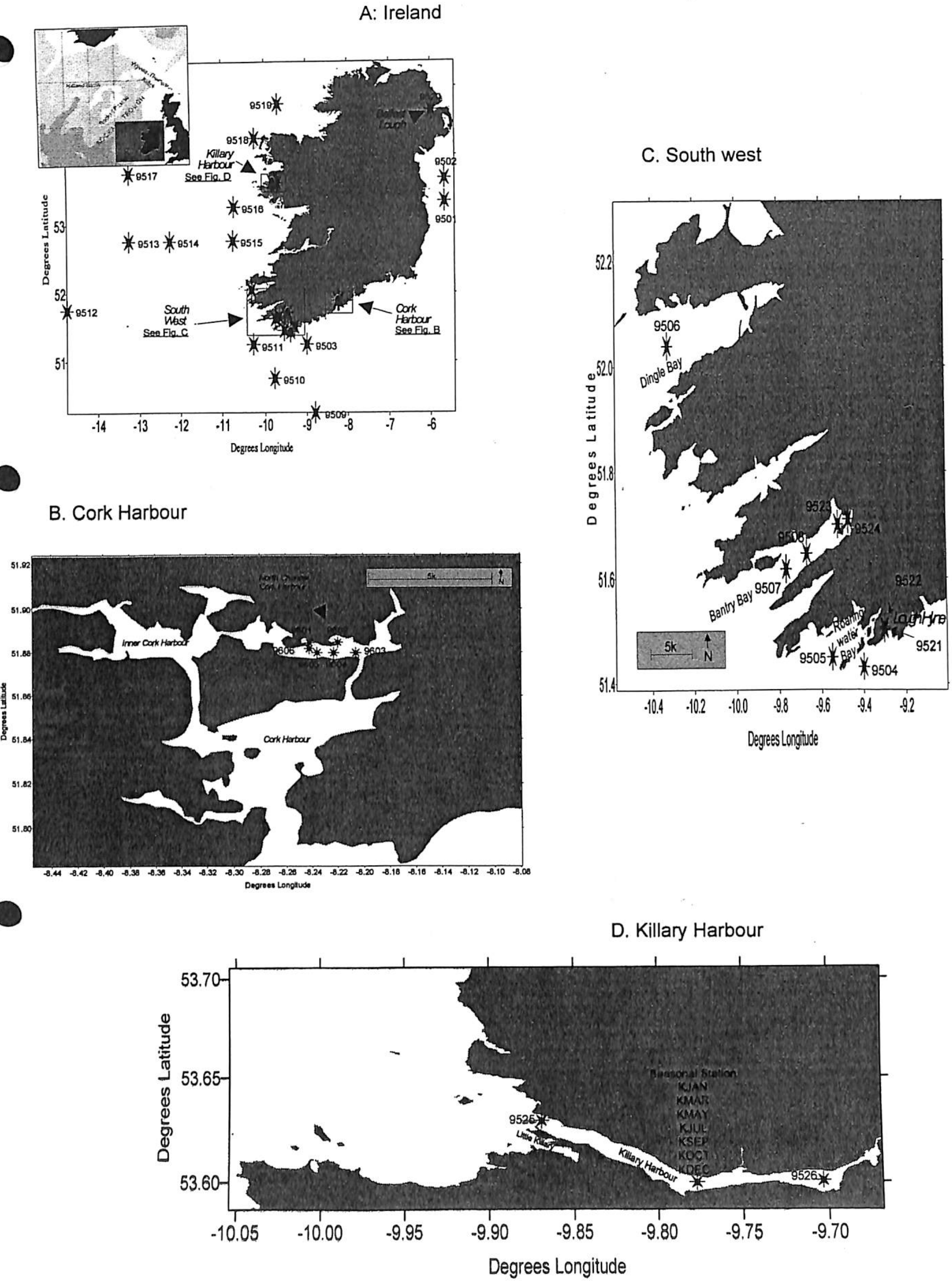


Fig 2: Sample locations 1995 -1997 cyst survey (a): Ireland, (b): Cork Harbour (c):South West and (d): Killary Harbour

Each location was sampled once, and these 32 sampling locations are shown in Figures 2 (a–d). Water samples from 4 shellfish production areas indicated in Figure 2 (a) (in red) were collected regularly between January 1995 and December 1997 to study the motile dinoflagellate species present.

Sediment samples for cysts were taken in waters ranging from 5 to 481m in depth and comprising fine mud to coarse shelly sand. They were taken during cruises aboard the R.V. Lough Beltra and R.V. Lough Foyle between May and July 1995. The samples were taken using a variety of grabs and corers depending on availability and sediment type and as far as possible the surface layer of the sediment was undisturbed when the grab was opened for sub-sampling.

Once onboard, the sediment was sub-sampled by gently inserting a 30mm diameter Perspex core tube into the surface layer of the sediment. The bottom of the tube was sealed off and withdrawn from the sediment. The sediment was carefully removed from the tube by pushing it out with a plunger from below. Samples were stored in the dark at 4°C. Samples were processed as outlined in Figure 3 to allow cysts to be observed under the light microscope.

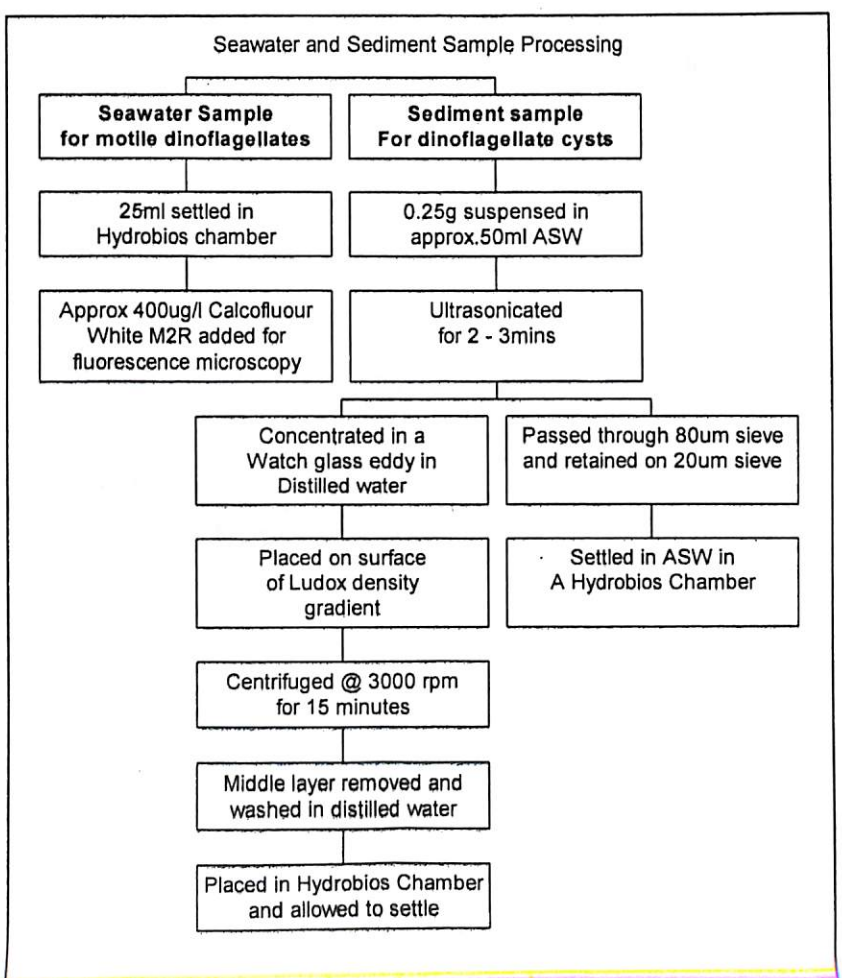


Figure 3: Sample processing stages.

In April 1999 a more detailed survey of Cork Harbour was carried out aboard the R.V. Celtic Voyager to attempt to observe the limits of *Alexandrium tamarense* cysts that were observed in the earlier scoping survey and thought to be responsible for the recurrent PSP problem in the area. The sites were chosen to give good geographical cover of the area and to try and obtain several types of sediment and grain sizes. These sites are shown in Figure 4.

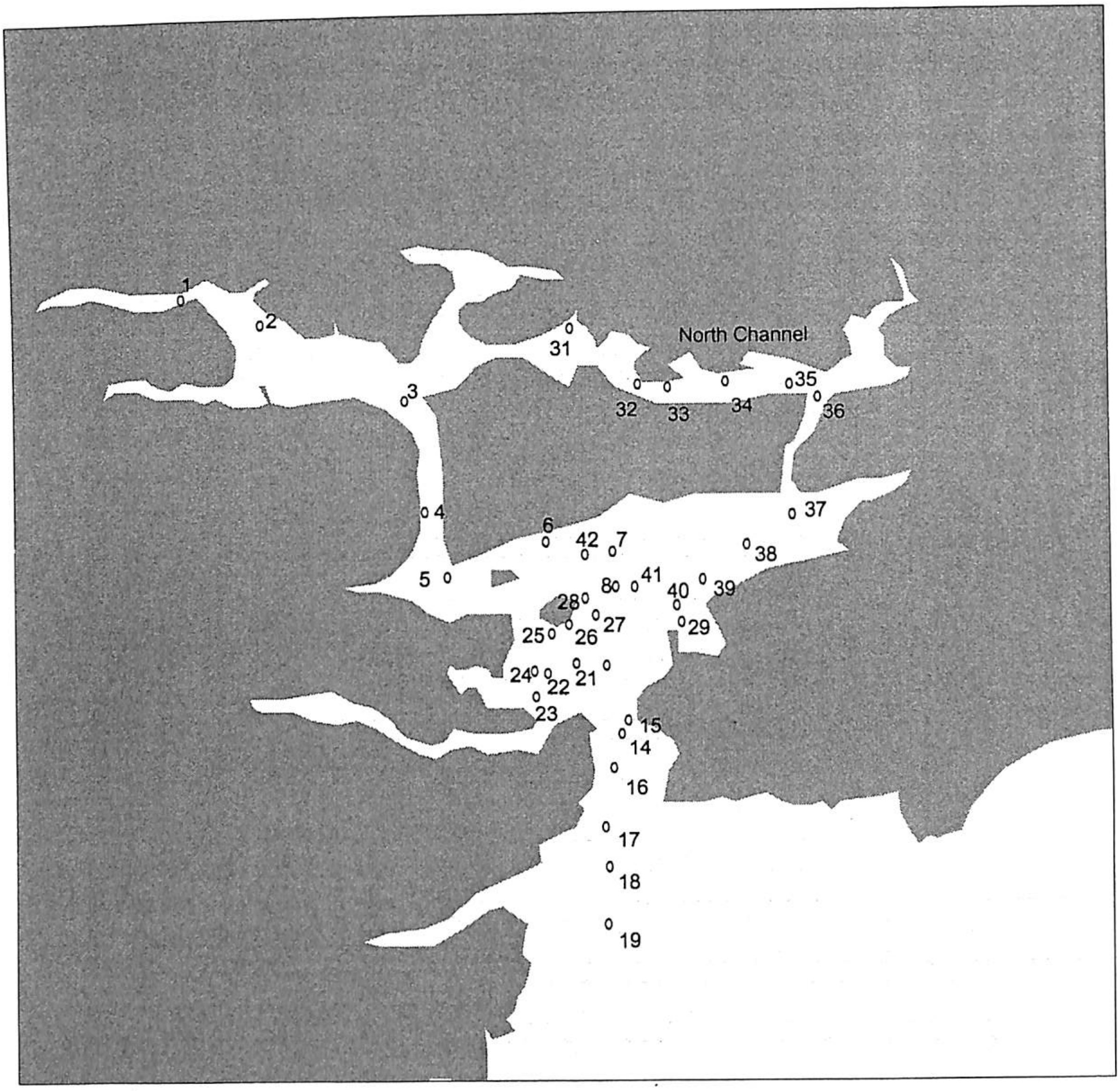


Figure 4: Site locations for detailed survey of Cork Harbour 1999

Samples were obtained using Reineck Corer and Van Veen grabs as outlined above and similar processing was carried out on the sediments prior to observation. Water depth ranged from 2m in the Western stations near Lough Beg, to over 50m in the outer stations.

Results:
In the 1995 – 1997 scoping survey 32 locations were examined. The sediments ranged from fine muddy silt in the North Channel of Cork Harbour to coarse sand in the Celtic Sea with the <62μm fraction content ranging from 1.6% in the coarse sediment (station 9510) to 98.9% in the fine sediment (station 9601). The moisture content ranged from 7.85% in the coarse sediments to 59.2% in the fine sediments. A discernible black anoxic layer was evident at a depth of 5cm in the two Irish sea samples (9501 and 9502) and also in the Bantry Bay sample (9523).

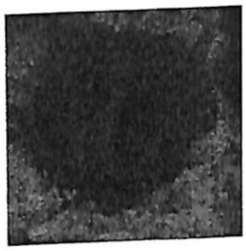
A total of 24 named species from 9 genera, 5 families and 2 orders were represented in the cysts recorded (Table 1). Selected species are illustrated in plate 1 (a-t).

Phylum: **Pyrrophyta.**		***Class:*** **Dinophyceae.**
Order	**Family**	**Genus**
Peridiniales:	Goniodomataceae:	
		Alexandrium
	Gonyaulacaceae:	
		Gonyaulax
		Lingulodinium
	Peridiniaceae:	
		Diplopelta
		Zygabikidinium
		Protoperidinium
	Calciodinellaceae:	
		Scrippsiella
Gymnodiniales:	Polykrikaceae:	
		Polykrikos
Uncertain:	Uncertain:	
		Polyasterias
		Cysts *indet.*
		Round Brown Cysts

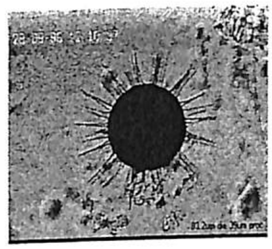
Table 1 Orders, families and genera of dinoflagellate cysts recorded.

All cysts, whether empty or full were recorded, and the viability of those cysts that had not hatched was not assessed. The number of species per sample ranged from 2 to 10. The results of a Pearson product-moment correlation showed no relationship with either the water content ($r = 0.1078$, $N=32$) or the percentage fine material in the sediment ($r = 0.0888$, $N=32$). In the upper 1cm, the most frequently recorded cyst was *Gonyaulax polyedra* which was present in 81.3% of the samples. The next most common species were *Scrippsiella trochoidea* (68.8%), Round Brown Cyst (65.6%), *Protoperidinium conicum* (59.4%) and *Polykrikos schwartzii* (34.4%). The 1cm and 5cm core sub-samples showed a similar top five species. The highest number of species recorded in any single location was at the station 50 miles west of Slyne head (9516). Ten species were recorded here in the surface 1cm sediment layer, although it was one of the lowest samples of fine sediment percentage at only 2.08%.

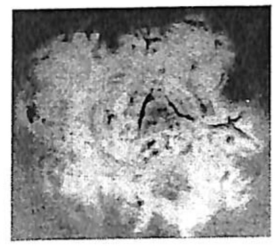
(a) *Alexandrium tamarense*



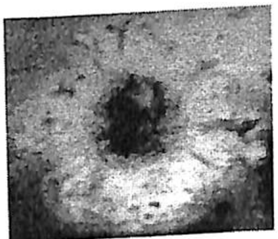
(b) *Diplopelta parva*



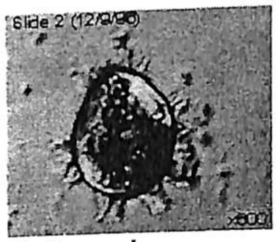
(c) *Gonyaulax polyedra*



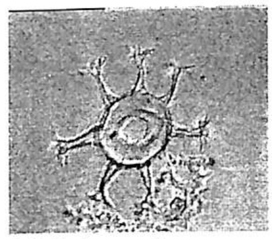
(d) *Gonyaulax spinifera* typ. *mirabilis*



(e) *Gonyaulax sp.*



(f) *Gonyaulax scrippsae*

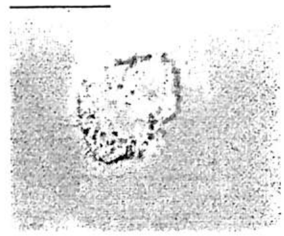


(g) *Polyasterias problematicus*

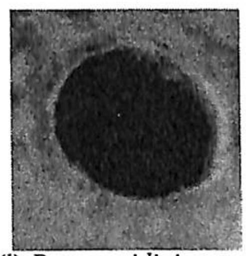
(h) *Polykrikos schwartzii*

(i) *Protoperidinium compressum*

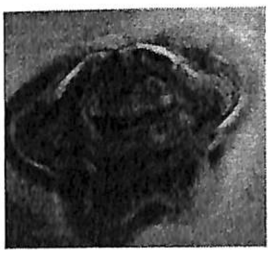
(j) *Protoperidinium conicum*



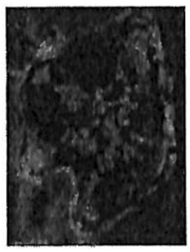
(k) *Protoperidinium claudicans*



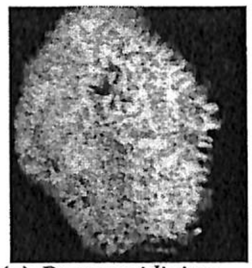
(l) *Protoperidinium excentricum*



(m) *Protoperidinium leonis*

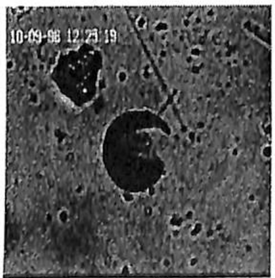


(n) *Protoperidinium oblongum*

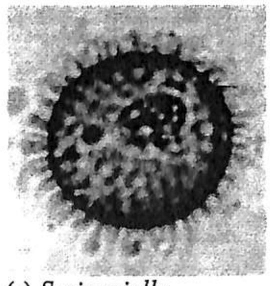


(o) *Protoperidinium pentagonum*

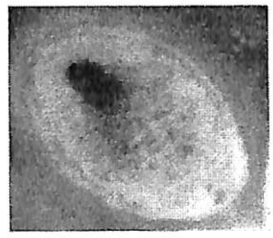
(p) *Protoperidinium subinerme*



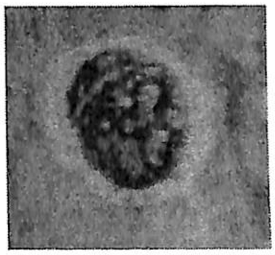
(q) *Protoperidinium thulesence*



(r) *Scrippsiella trochoidea*



(s) *Scrippsiella lachrymosa*



(t) *Preperidinium meunieri*

Plate 1 a - t: Dinoflagellate cysts, isolated from sediments during the investigation.

	Cyst	Motile
Alexandrium tamarense (Lebour) Balech	x	X
Ceratium extensum (Gourret) Cleve		X
Ceratium furca (Ehrenberg) Claparede & Lachmann		X
*Ceratium fusus (*Ehrenberg) Dujardin		X
Ceratium inflatum (Kofoid) Jorgenson		x
*Ceratium lineatum (*Ehrenberg) Cleve		x
Ceratium longirostrum Gourret		x
Ceratium teres Kofoid		x
Ceratium tripos (O.F. Muller) Nitzsch		x
Species *Indet.*	x	x
Dinophisis ovum Schutt		x
Dinophisis sacculus Stein		x
Dinophysis acuminata Claparede & Lachmann		x
Dinophysis acuta Ehrenberg		x
Dinophysis rotundata Claparede & Lachmann		x
Diplopelta parva (Abé) Matsuoka	x	
Diplopsalis sp Bergh		x
Gonyaulax polyedra (Stein)	x	x
Gonyaulax scrippsae Kofoid	x	
Gonyaulax sp Deising	x	x
Gonyaulax spinifera typ. *elongatus* Reid	x	
Gonyaulax spinifera typ. *mirabilis* (Rossignol) Sarjaent	x	
Gonyaulax verior Sournia	x	
Gymnodinium simplex (Lohman) Kofoid & Swezy		x
Gymnodinium sp Stein		x
Gyrodinium aureolum Hulbert		x
Gyrodinium fusiforme Kofoid & Swezy		x
Gyrodinium glaucum (Lebour) Kofoid & Swezy		x
Gyrodinium lachryma (Meunier) Kofoid & Swezy		x
Gyrodinium prunus (Wulff) Lebour		x
Gyrodinium sp Kofoid & Swezy		x
Gyrodinium spirale (Bergh) Kofoid & Swezy		x
Helgolandinium subglossum von Stosch		x
Heterocapsa sp Stein		x
Heterocapsa triquetra (Ehrenbergh) Stein		x
Katodinium glaucum (Lebour) Loeblich		x
Polyasterias problematicus Meunier	x	
Polykrikos schwartzii Chatton	x	x
Preperidinium meunieri (Pavillard) Elbrachter	x	
Prorocentrum gracile Schütt		x
Prorocentrum micans Ehrenberg		x
Prorocentrum minimum (Pavillard) Schiller		x
Prorocentrum scutellum Schröder		x
Protoperidinium bipes (Paulsen) Balech		x
Protoperidinium brevipes (Paulsen) Balech		x
Protoperidinium claudicans (Paulsen) Balech	x	x
Protoperidinium compressum (Abé) Balech	x	
Protoperidinium conicum (Gran) Balech	x	
Protoperidinium denticulatum (Gran & Braarud) Balech	x	
Protoperidinium depressum (Baillie) Balech		x
Protoperidinium excentricum (Paulsen) Balech	x	x
Protoperidinium leonis (Pavillard) Balech	x	
Protoperidinium minutum (Kofoid) Loeblich	x	x
Protoperidinium oblongum (Aurivillius) Parke & Dodge	x	
Protoperidinium ovatum Pouchet		x
Protoperidinium pellucidum Bergh		x
Protoperidinium pentagonum (Gran) Balech	x	x
Protoperidinium punctulatum (Paulsen) Balech		x
Protoperidinium pyriforme (Paulsen) Balech		x
Protoperidinium sp Bergh	x	x
Protoperidinium steinii (Jorgenson) Balech		x
Protoperidinium subinerme (Paulsen) Loeblich	x	
Protoperidinium thulescence (Balech) Balech	x	
Round Brown Cyst	x	
Scrippsiella lachyrmosa Lewis	x	
Scrippsiella sp Balech		x
Scrippsiella trochoidea (Stein) Loeblich	x	x

Table 2: List of the species recorded in the course of the scoping study 1995 –1997

The combined cyst distribution data were analysed using cluster analysis. This determined seven groups or clusters of sample sites at the 10% level of similarity. This means that these groups formed branches of the dendrogram that were at least 90% dissimilar to each other. The graphic is shown in figure 5 and the groupings are mapped in figure 6.

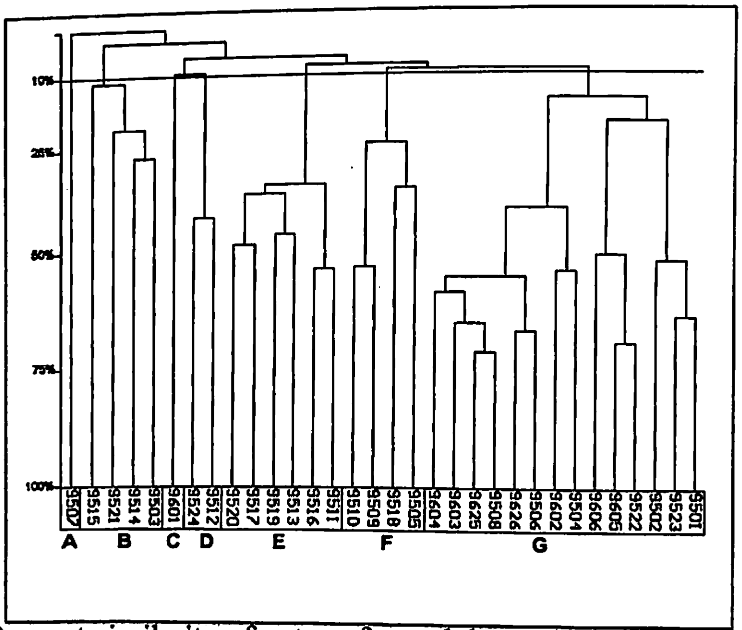


Figure 5: Percent similarity of untransformed data using the average pair group clustering method, the clusters at 10% similarity are indicated

The groups were described as the following broad groupings based on cyst counts:

A	Bantry
B,D,E,F	Open Water Sites
C	Cork Harbour
G	Irish Sea and Inshore sites

One of the Bantry sites and one in Cork Harbour formed distinct branches on the dendrogram being is clearly separated from the other station groupings at a much lower level of similarity. Groups B, D, E and F comprised of groups that included all of the offshore sites but also included some inshore sites, while group G consisted of the Irish sea sites and the remaining inshore sites.

The assemblage of cysts in the Bantry site (9507) was sufficiently different to the other groups to form a single branch on the dendrogram. This is explained by the dominating presence of *Gonyaulax polyedra* cysts in the sample. When the occurrence of the motile forms of this species were investigated from the database of phytoplankton records at Fisheries Research Centre it is apparent that this species forms rather sporadic blooms in the area.

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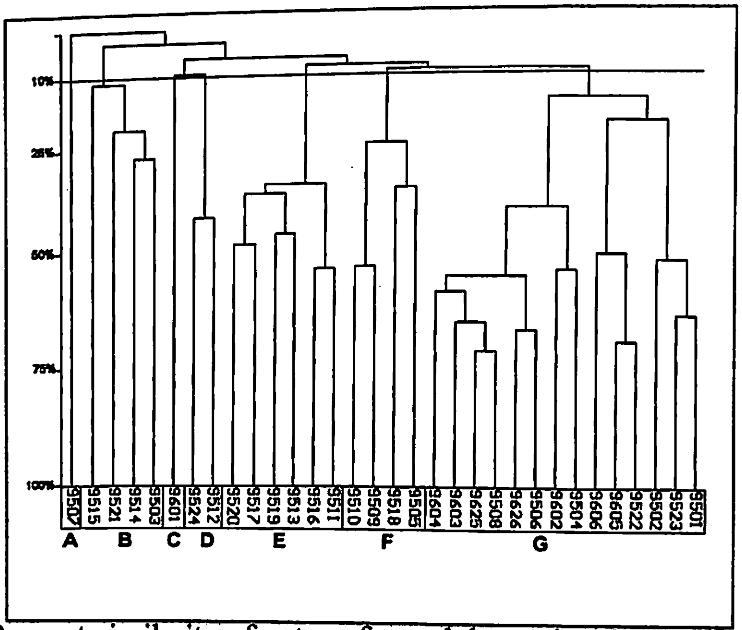

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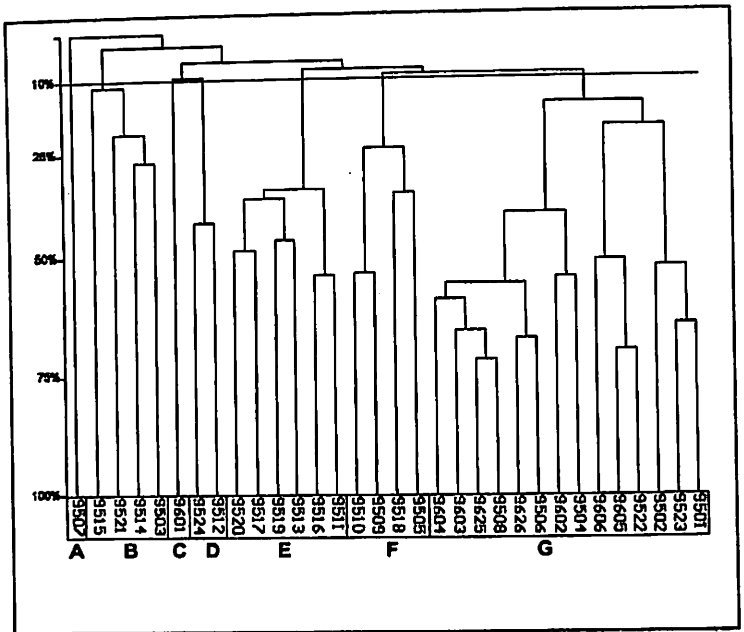

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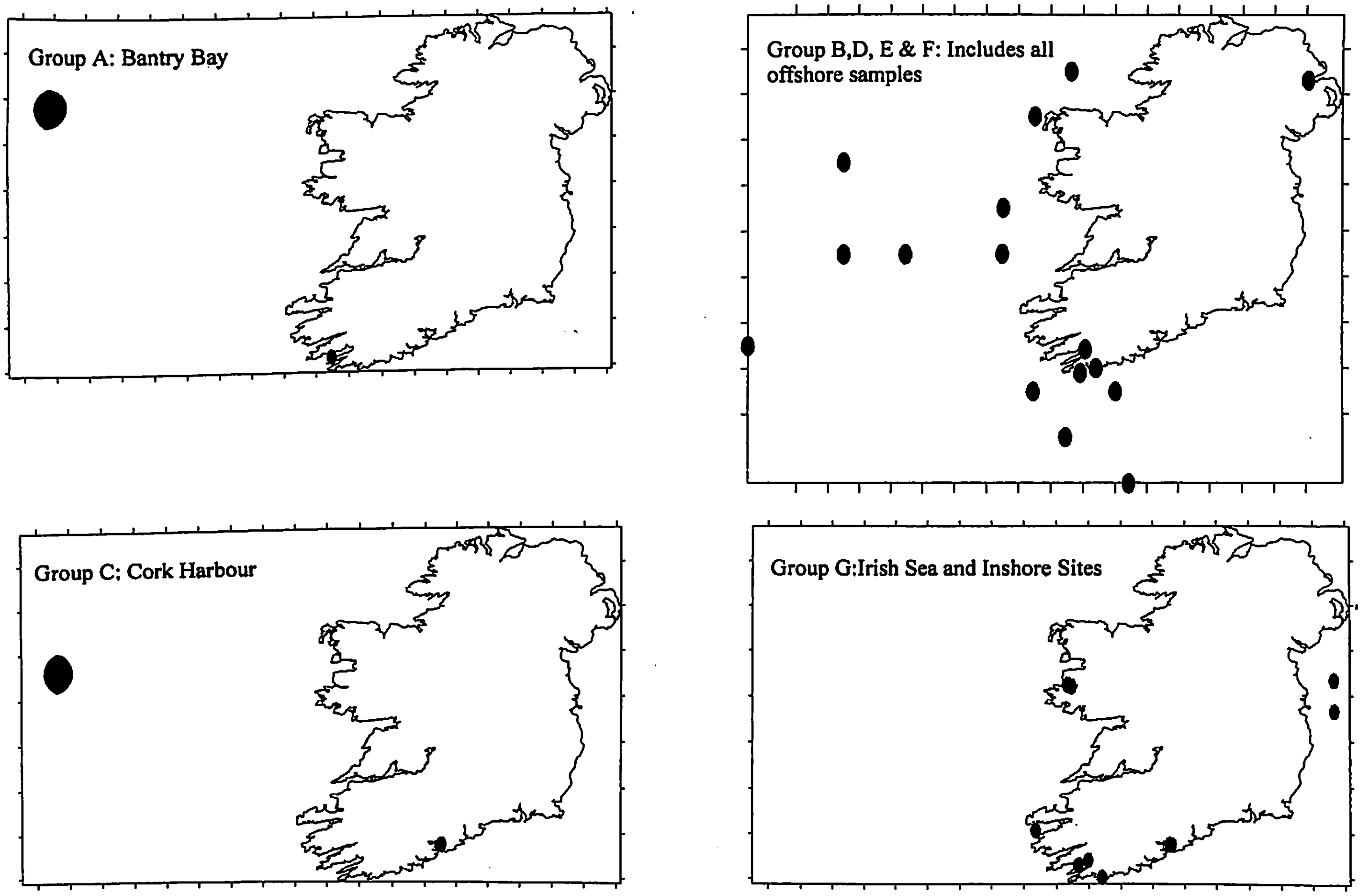

Fig 6: Location of clusters at 10% similarity as indicated in fig. 5

Station 9601, another single location group on the dendrogram, was located within Inner Cork Harbour on the North Channel. This location was different from the other locations due to the presence of *Alexandrium tamarense* cysts at moderate densities of up to 1147 cysts.g^{-1}. *Gonyaulax spinifera* typ. *mirabilis* and Round Brown Cysts were also present between 2000 and 3000 cysts.g^{-1} in this location. While these species were found elsewhere, the Cork samples held the highest counts.). In this area *Alexandrium tamarense* cysts were observed in sediments at four sites, these were the only records of this species in this study. The cysts were found at higher densities in the surface 1cm sediment layer than the 5cm layer suggesting that the 1996 bloom of the species may have contributed to the cyst assemblage in the sediment. These surface cysts formed a small but important constituent of the population. As they were limited to two locations it is likely that they followed a very localised bloom. The 5cm sediment cores in this area had *Alexandrium* present in 4 out of 6 samples. This suggests that a wider distribution is present, and that previous blooms were more extensive. As *A. tamarense* often does not show in the phytoplankton of this area, sometimes for many years (Fig 7), it is very likely that the presence of cysts in the sediment is ecologically important to its continuation.

The results from the 1999 detailed survey of cork harbour confirmed the presence of *Alexandrium tamarense* cysts (Plate 2), and also showed that the distribution is limited to the North Channel of the Harbour. The concentrations ranged from 20 to 600 cysts per dry gram of sediment and they were present in the 6 samples taken in the North Channel. Slurries of these sediments yielded viable vegetative cells thereby confirming the identification.

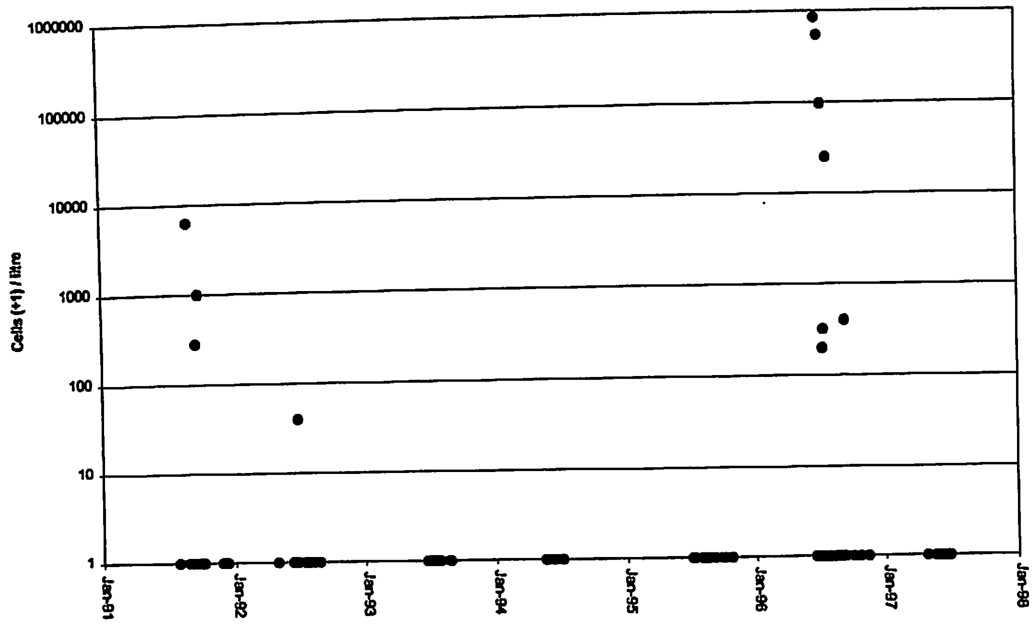

Fig 7: Time series 1991 –1997 *Alexandrium tamarense* in summer samples of phytoplankton from Cork harbour

Discussion:

The mapping of dinoflagellate cysts in marine sediments has been shown to be a useful tool to indicate areas where dinoflagellate blooms have occurred in the past or may occur in the future. The distribution of dinoflagellate cysts in Irish coastal waters was investigated to determine what species of dinoflagellate cysts were present, and to see where they were abundant. As the occurrence of dinoflagellate blooms is of great importance to aquaculture and fisheries in Ireland, this present study concentrated on areas of important aquaculture production. In addition, further samples were taken from offshore sediments to observe any difference between these and the inshore samples.

This study showed a seed bed of *Alexandrium tamarense* cysts is present in the North Channel of Cork Harbour. Moderate concentrations of cysts were found following PSP causing blooms in the North Channel. The motile form was also detected in Killary Harbour and Roaringwater Bay at low levels but no cysts were found at these locations indicating that the species has not established a seed bed in these locations. The presence of the cysts of *Alexandrium tamarense* in Cork Harbour is notable because it means that recurrent blooms are likely with resulting closures to the resident shellfish farms.

With the advent of EC Council Directive 91/67/EEC the free movement of shellfish into Ireland commenced in 1993. This raised some serious implications for the movement and introduction of non-native dinoflagellates from continental Europe. When shellfish are harvested there may be associated species including dinoflagellate cysts in the mud around and within the shells, as well as within the gut. This may be a means by which phytoplankton species may be introduced into other areas.

O'Mahony (1993) examined half-grown Pacific oysters (*Crasssostrea gigas*) that were imported into Ireland for the presence of non-native flora. These were imported for relaying and on-growing. Samples of gut content and sediment from the shellfish were found to include 22 dinoflagellate species and 15 species of dinoflagellate cysts were found.

Dijkema (1992) calculated that in the Netherlands 2.5 million cysts of toxic dinoflagellates may be present in one tonne of imported mussels originating from an area affected by a red tide. He went on to show that the quantity of dinoflagellate cysts imported in shellfish into Dutch coastal waters are of the same order of magnitude as are imported through ballast water into Australia annually. The Dutch have placed a ban on the immersion into coastal waters of any imported bivalves to prevent the inadvertent introduction of any nuisance species.

Toxic phytoplankton are obviously now regarded as potential nuisance and the movement of shellfish species in the course of trade may result in the transfer and establishment of exotic species. This may hinder the sale of shellfish into countries that have imposed criteria regarding the immersion of imported consignments. The movements between different biological provinces may result in as yet unknown ecological consequences.

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